CHEMISTRY AND MICROBIOLOGY OF THE LITTER OF SOME FOREST TREES

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ABSTRACT

The differences in the composition of various groups of lipophilic extractives, low-molecular carbohydrates, cyclitols, phenolic glycosides, polysaccharides, lignin, ash and crude protein in green needles and brown needles (on the tree as well as in litter form after various length of time) from pine (Pinus sylvestris) has been studied. Work in progress on the effect of extracts from these sources as well as from needles from spruce (Picea abies) and aspen (Populus tremula) on some litter-decomposing and mycorrhizal fungi is also discussed.

INTRODUCTION

The intention of the present paper is to discuss briefly some recent developments and studies in progress at the Swedish University of Agriculture, Forestry and Veterinary Medicine, in the field of chemistry (partly in cooperation with K. Hannus at Åbo Akademi Finland) and microbiology of the litter of some Swedish forest trees. This communication is not intended as a summary of the field but represents mainly glimpses from our own activities. At present the chemical results exceed the microbiological results and they are mainly concerned with pine needles.

Until recent years interest in the chemical composition of the foliage part of the forest trees (needles or leaves, twigs and slender branches) has, in comparison with other parts of the tree, been rather neglected[1]. This is still true concerning the changes in the foliage part after it has fallen to the ground or logging residues left in the forest to form the litter. In recent years the attention has been focussed on a better utilization of the tree biomass. The foliage, which is very rich in extractives, is not generally desired in the pulping processes as it increases the chemical consumption during the cook. For a more effective

utilization the foliage is therefore separated from the chips by some screening process. The separated fraction, mainly consisting of needles or leaves, offers considerable potential for the production of chemicals or use as a valuable animal feed constituent after grinding and steam treatment (so called muka). Such uses were developed in the Soviet Union many years ago[2], but in recent years there has also been a growing interest in the field in North America and Scandinavia.

For better utilization of this material, a good knowledge of the chemical composition is most desirable. As a high proportion of, for instance, the needles on pine and spruce trees are brown (around 25 % or less) it is also of interest, apart from the biochemical and physiological aspects, to compare the composition of these dead needles with the green ones. A better understanding of the chemistry of dead needles and leaves and their further decomposition would in particular be important in connection with problems concerning the accumulation and decomposition of organic matter in the soil and its microbiological activity.

The present paper discusses the differences in the chemical composition between green and brown needles (on the tree as well as in litter form after various lengths of time) from **Scots** pine (Pinus sylvestris), work in progress on the chemistry of needles from Norwegian spruce (Picea abies) and preliminary studies on the effect of extracts from these sources and leaves from aspen (Populus tremula) on some litter-decomposing and mycorrhizal fungi.

CHEMICAL COMPOSITION OF GREEN PINE AND SPRUCE NEEDLES

There are very few investigations, which cover more than one or a few groups of needle constituents and it is also obvious that seasonal, environmental and individual variations and probably also storage and treatment conditions may cause considerable variations of the chemical composition of the needles. Therefore reports by different workers can present divergent results.

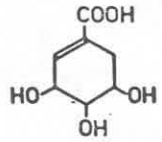
In recent years, extensive investigations on the lipophilic extractives of the so called technical foliage from **Scots pine** (Pinus sylvestris) have been reported by Hannus and Pensar (for a summary, see ref. 1). The investigated product consisted of 60-70 % needles and 30-40 % wood parts. In connection with our investigation, soon to be completed, on the composition of brown pine needles on the tree and after different times as litter, (discussed below[3]) we also studied the lipophilic extractives in pure, green needles (see Tables 1-4).

We have been interested in the constituents of conifer needles for many years and the studies initiated at the Swedish Forest Research Laboratories[4,5] have continued during recent years in Uppsala. Among the hydrophilic extractives (water-soluble part of an acetone extract) - mainly carbohydrates and the related cyclitols - some compounds such as glucose, fructose, sucrose, pinitol and

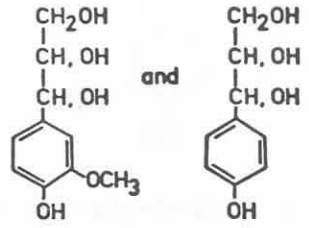
shikimic acid were isolated in a yield of 1.5 - 2.5 % of dry weight each. Also present (in the range of 0.1 - 1 %) were arabinose, rhamnose, mannitol, melibiose, raffinose, myoinositol and sequoitol and also traces of laminaribiose and cellobiose.

In the hydrophilic part of the acetone extract, a complex mixture of phenolic glycosides and phenols was found. They can be enriched from the main carbohydrate extractives by extraction of the aqueous solution with n-butanone and for a first crude fractionation we generally use chromatography on a Sephadex LH-20 column. The order of elution of the main groups of compounds (or individual compounds) are given in Fig. 1. The pure compounds are obtained by chromatographic subfractionations on silicic acid or ion-exchange columns, preparative thin-layer chromatography or high-pressure liquid chromatography. After the group of monocyclic glycosides (of guaiacyl- and p-hydroxyphenylglycerol) we obtained a complex mixture of dilignol glycosides, which we have recently fractionated and identified[6,7] (see Fig. 2). In the former group of glycosides, which are also present in other parts of the tree and are of interest in connection with the discussion of the biosynthesis of lignin, glucose is linked to hydroxyl groups in the glycerol side chain[5]. The elucidation of some of those compounds is still in progress. For a summary of methods for the isolation and identification of the phenolic glycosides, see ref. 6. dihydrog quercetin-3'-β-glucoside (sometimes around 2% of dry weight), followed by compound 2 (ca. 0.4 %), 3 (ca. 0.3%), 1 and 4 (ca. 0.2 % of each) are the predominant glycosides.

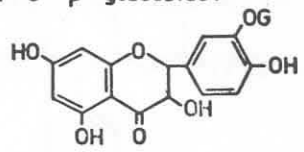
1. Mono- and oligosaccharides, sugar alcohols, cyclitols
2. Shicimic acid:



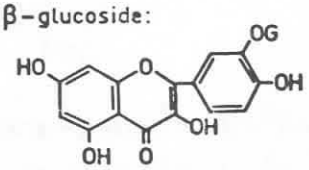
3. β-Glucosides of:



4. Dilignol glycosides
5. Dihydroquercetin-3'-β-glucoside: (Taxifolin)



6. Quercetin-3'-β-glucoside:



7. Dihydroquercetin
8. Quercetin
9. (+)-Catechin:

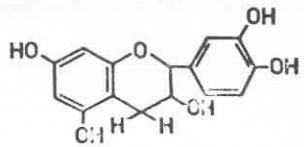
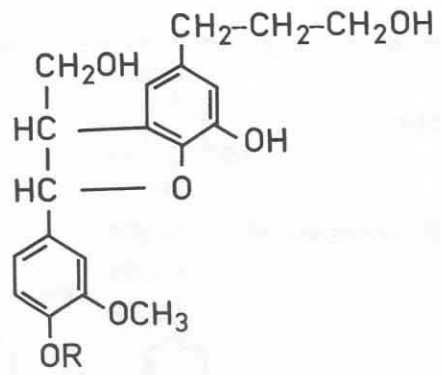


Figure 1. Fractionation of 2-butanone extract from green pine needles on Sephadex LH-20 (eluent: water and aqueous ethanol).

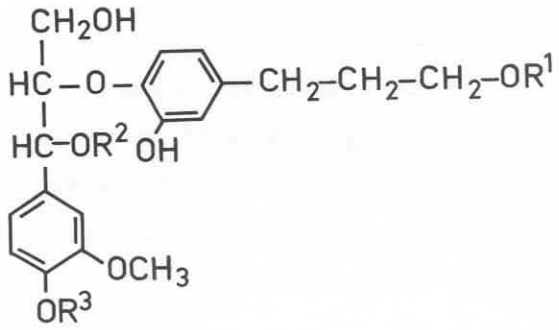
In the lipophilic part of the acetone extract (soluble in methylene chloride), the main acid constituent (1 - 2 %)[9] consisted of a diterpene acid, named pinifolic acid. A corresponding dehydro acid as well as benzoic acid were later identified amongst the other acid components[10].

In the studies of brown and litter needles, we have investigated the chemical composition of the residue of green needles after



1 R = β-D-glucopyranoside

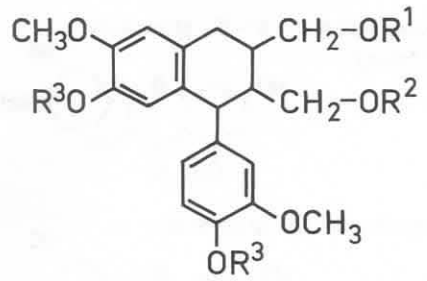
2 R = α-L-rhamnopyranoside



3 $R^1 = R^3 = H$ R^2 = β-D-glucopyranoside

4 $R^1 = R^2 = H$ R^3 = β-D-xylopyranoside

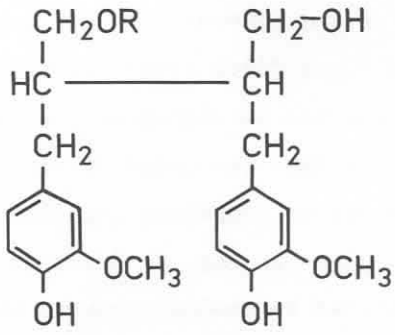
5 $R^2 = R^3 = H$ R^1 = α-L-rhamnopyranoside



6 $R^1 = R^3 = H$ R^2 = α-L-arabinofuranoside

7 $R^2 = R^3 = H$ R^1 = β-D-glucopyranoside

8 $R^1 = R^3 = H$ R^2 = β-D-glucopyranoside



9 R = β-D-glucopyranoside

Figure 2. Dilignol glycosides isolated from green pine needles.

the acetone extraction by recording the amount of acidic (uronic acid) and neutral polysaccharides, Klason lignin, crude protein and ash content (Table 4). For comparison the corresponding figures for needles from Norwegian spruce (Picea abies) are given.

The lipophilic extractives from spruce needles have not been studied as extensively as those from pine needles. There are some reports on phenolic compounds. Thus more recently Medvedeva et al.[11] reported a series of flavonoid glycosides and phenolic acids from Siberian spruces. We are at present studying the hydrophilic extractives from the needles of Picea abies[12] and in addition to compounds previously found in spruces, the glycosides 1, 2 and 3 (Fig. 2), which we previously found in pine needles[6,7], have also been isolated. It is notable that we also found a glucoside corresponding to compound 1 but with both phenolic groups methoxylated. Pinifolic and dehydropinifolic acids, previously found in pine needles[9,10], were not found. Thompson et al.[13] have studied procyanidins (catechins) in Pinus sylvestris and Picea abies.

STUDIES ON BROWN PINE NEEDLES FROM TREE AND IN THE LITTER

In an investigation[3] (now nearly completed), connected with the large Swedish Coniferous Forest Project[14], we have studied the chemical composition of brown needles from Pinus sylvestris and then followed the chemical changes after different times on the ground. The four year old needles (sample B below) were picked from 15 year old trees, from the experimental site at Jädraås, Province of Gästrikland, Sweden, in October just before the time for falling. Parts of the sample were stored for different times on the forest ground in bags of nylon net (samples C, D and E for 3, 6 and 12 months respectively. The drop in dry weight of these three samples during the storage was 10.4, 17.8 and 27.3 % respectively. The yields of various fractions and components given in the Tables, however, are given on the actual dry weight of the particular sample. Also investigated was a sample of green pine needles (called sample A), collected in May from the same trees. For comparison, some data from green needles of Picea abies, collected in winter, are also given.

The samples were extracted for 2 x 0.5 h with boiling acetone. After filtration, the needles were dried, milled and extracted first with acetone (2 x 0.5 h) and then 50 % aqueous acetone (2 x 0.5 h) in an ultra-sound bath at room temperature. The combined extracts were evaporated to a small volume and by consecutive extractions divided into fractions comprising compounds soluble in light petroleum, ethyl acetate, 2-butanone (saturated with water) and water. The yields of extracts obtained by evaporation at reduced pressure (without further drying at elevated temperatures) are given in Table 1. Also given are dry residues after extraction.

In the series A→E, one can notice a steady decrease in the amount of the lipophilic (light petroleum) and n-butanone extractives but an increase in the ethyl acetate extractives. The water extractives drop considerably going from the green to the brown needle on the tree and their amount then reaches a maximum in the litter stage. There is a drop in the amount of non-extractives from green to brown needles but the amount of those residues is then fairly stable.

The amounts of the major groups of compounds in the light petroleum extractives (hydrocarbons, steryl esters, triglycerides, free acids and free diterpene alcohols plus sterols) are given in Table 2. The determination of the individual compounds in the groups is in progress. Mono-terpenes probably disappear during the procedure used and pinoprenyl acetates are not determined. There is a notable drop in the amount of steryl esters and triglycerides from green to brown needles on the tree followed by a further decrease in the litter. The amount of free acids is fairly stable and hydrocarbons and alcohols drop slowly. Amongst the steryl esters palmitic (16:0), oleic (9, 12 - 18:2), 5, 9, 12 - 18:3- and 9, 12, 15 - 18:3 acids are dominant constituents and in the triglycerides these acids plus 9 - 18:1 acid.

Amongst the free acids, the diterpene acids dominate, with pinifolic acid as the main component (ca. 0.6 % of dry weight in all samples). β-Sitosterol and a diterpene alcohol of the labdane type (under elucidation[15]) are dominant components amongst the free sterols and alcohols respectively.

In the ethylacetate extracts are found aglycones of dilignol glycosides, dihydroquercetin, quercetin and procyanidins (catechins), but the amount of identified compounds decreases going from green to brown needles and further decreases are noted with the increased time as litter. The amount of more condensed products increases steadily. The phenolic glycosides are enriched in the n-butanone extract, and most of the low-molecular carbohydrates and cyclitols are found in the water extracts. The amounts of some of the identified compounds in these three extracts are given in Table 3. Usually we find higher values of dihydro quercetin-3'-β-glucoside (ca. 2%) than in sample A. There is a drastic drop in the amount of sugars and cyclitols going from green to brown needles. The small amounts of glucose and mannitol found in the litter even after one year might mainly be resulting from microbial polysaccharide degradation and synthesis.

The amounts of ash, crude protein (6.25 x N-value), polysaccharides (neutral and acidic parts) and Klason lignin in the residues after extraction were determined by conventional methods and are given in Table 4. The ash-contents are fairly stable and there is a drop in the crude protein-value going from green to brown needles. An increase in the latter value of the litter with time might indicate production of microbial protein and chitin. The amounts of polysaccharides in the different needle samples do not differ in a characteristic way but the increasing content of Klason lignin with time shows a distinct trend. The lignin values also represent some nitrogen containing compounds. Cellulose is clearly the predominating polysaccharide component followed by mannose-containing hemicelluloses.

Extract	Pine needle samples A	B	C	D	E	Spruce needles
Light petroleum	12.2	8.4	6.4	5.7	4.9	10.3
Ethyl acetate	5.0	6.5	9.7	9.8	9.4	3.1
2-Butanone	4.0	3.1	0.9	-	-	4.6
Water	19.9	9.2	13.1	12.5	7.9	27.1
Residue	65.4	73.2	76.7	79.6	78.5	58.7

Table 1. Amounts of extractives and residues from pine and spruce needles (given as percentage on dry weight of the needles).

Group of compounds	Pine needle samples A	B	C	D	E
Hydrocarbons	0.3	0.2	0.2	0.1	0.1
Steryl esters	1.2	0.6	0.5	0.3	0.2
Triglycerides	2.8	0.7	0.2	0.2	0.1
Free acids	1.1	1.2	1.0	1.2	1.0
Diterpene alcohols plus sterols	0.9	0.7	0.5	0.4	0.4

Table 2. Amounts of major group of lipophilic extractives from pine needles (given as percentage on dry weight of the needles).

Compound	Pine needle samples A	B	C	D	E
Glucose	2.3	0.4	0.3	0.2	0.1
Fructose	1.6	0.5	tr	-	-
Sucrose	0.7	0.2	tr	-	-
Mannitol	1.8	0.1	0.2	0.1	0.1
Pinitol	2.2	0.4	0.1	0.1	tr
Myoinositol	0.3	tr	tr	tr	tr
Glycoside 2	0.3	0.4	tr	tr	-
Dihydroquercetin-3'--β-glucoside	0.3	0.3	tr	tr	-

Table 3. Amounts of some hydrophilic extractives from pine needles (given as percentage on dry weight of the needles; tr = traces.

	Pine needle samples A	B	C	D	E	Spruce needles
Ash	1.6	1.5	1.5	1.4	1.6	3.0
Crude protein	5.3	2.4	2.5	2.9	3.6	5.9
Acidic polysaccharide constituents	3.3	4.6	4.1	4.3	4.0	3.2
Neutral polysaccharide constituents, calculated as:						
Glucans	24.8	19.4	23.4	28.5	25.1	16.3
Mannans	4.4	5.9	6.7	7.9	7.5	7.5
Galactans	1.9	2.8	3.1	3.0	2.7	1.5
Arabinans	2.6	4.0	3.0	2.5	2.1	3.1
Xylans	1.3	2.1	2.2	2.1	1.9	2.1
Klason lignin	14.8	22.6	25.7	28.4	31.3	14.4

Table 4. The amounts of ash, crude protein, polysaccharides and Klason lignin in the residues after extraction (given as percentage on dry weight of the needles).

STUDIES ON THE EFFECT OF EXTRATIVES FROM PINE AND SPRUCE NEEDLES AND ASPEN LEAVES ON THE GROWTH OF FUNGI

In cooperation with the Department of Forest Botany, The Swedish College of Forestry (G. Lindeberg) and the Department of Microbiology, The Agricultural College of Sweden (B. Berg) we have studies in progress on the effect of extracts from pine (Pinus sylvestris) and spruce (Picea abies) needles and from aspen (Populus tremula) leaves on forest soil fungi (litter-decomposing and mycorrhizal) and also on some parasitic fungi.

Preliminary tests with the different type of extracts from the brown pine needle sample B (above) indicated a growth-promoting effect on the litter-decomposers Marasmius androsaceus and scorodonius of the water extract, compared with a glucose containing control. The ethyl acetate extract showed an inhibiting effect on the latter fungus as well as on Trichoderma viride, polysporum and Cladosporum herbarum. The mycorrhizal fungus Boletus variegatus was inhibited by the ethyl ecetate extract but somewhat stimulated by the water extract. The pine parasite fungus Lophodermium pinastri was strongly stimulated by all four extracts.

The effect of the addition of different amounts of solutions of extracts from green spruce needles (containing concentrations of extracts in proportion to their contents in the needles) on the growth of the typical spruce needel-decomposing Marasmius perforans and the spruce parasite Scleroderris lagerbergii is illustrated in Figure 3. A strong stimulating effect of the water extract on the growth of both fungi is obvious and

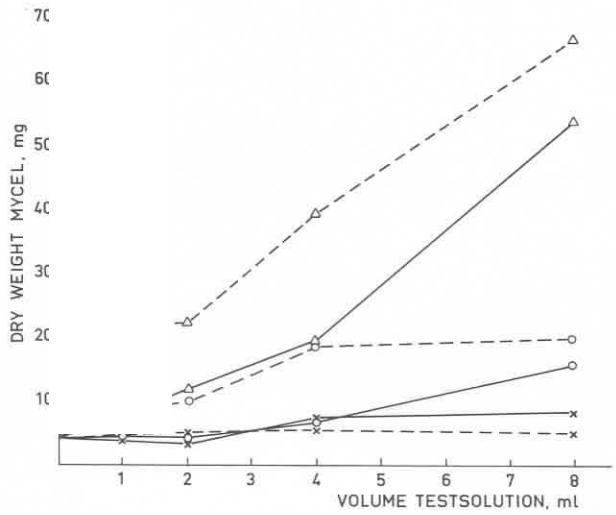


Figure 3. Effect of water (△), ethyl acetate (x) and light petroleum (o) extracts from spruce needles on the growth of Marasmius perforans (---) and Scleroderris lagerbergii (——).

also some stimulation from the light petroleum extract is indicated.

Olsen et al.[16] have previously found that both green and yellow leaves of aspen (Populus tremula) contain a water-soluble factor, which strongly stimulates the growth of litter-decomposing Marasmius fungi, but also ethyl acetate soluble compounds (benzoic acid and catechol), strongly inhibiting the growth of different mycorrhizal Boletus-species, but less inhibiting the Marasmius-species. We are at present trying to isolate the active compound(s) in the growth-stimulating factor.

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